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EXPERIMENTAL STUDIES

Limitation of No Reflow Injury by Blood-Free Reperfusion With Oxygenated Perfluorochemical (Fluosol-DA 20%)

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This study was designed to assess the effects of blood-free reperfusion with oxygenated or unoxygenated intracoronary perfluorochemical (Fluosol-DA 20%) on myocardial perfusion and to determine its mechanism or mechanisms of limiting no reflow. Twenty-four dogs underwent 90 min of coronary occlusion followed by 210 min of reperfusion and were randomized to either: 1) blood-free reperfusion with intracoronary oxygenated perfluorochemical (20 ml/kg per min) for 20 min followed by blood reperfusion (n = 8); 2) intracoronary unoxygenated perfluorochemical administered as in those treated with oxygenated perfluorochemical (n = 8); and 3) blood reperfusion alone (control) (n = 8). Regional myocardial blood flow was serially determined and global myocardial perfusion was assessed by an intravenous injection of the fluorescent dye (thioflavin-S). Quantitative studies were performed to determine neutrophil infiltration and extent of endothelial injury.

Hemodynamic variables were similar in all groups. The zone of impaired perfusion (thioflavin negative), expressed as a percent of the left ventricle, averaged 10 ± 2%, 6 ± 2% and 3 ± 1%, in control and unoxygenated and oxygenated perfluorochemical groups, respectively (control versus oxygenated perfluorochemical $p < 0.004$). The reduction in thioflavin-negative area with oxygenated perfluorochemical was associated with a notable recovery of endocardial blood flow (0.97 ± 0.22 vs. control 0.39 ± 0.08 ml/min per g; $p < 0.04$) at 210 min of reperfusion. The number of capillaries plugged by neutrophils (per 200 capillaries) in thioflavin-negative areas was similar with both oxygenated (5.9 ± 1.4) and unoxygenated perfluorochemical (5.4 ± 0.8) treatment and was significantly less than that with the control group (18.9 ± 3.2, $p < 0.003$).

Myocardium reperfused with oxygenated perfluorochemical demonstrated significantly greater preservation of endothelial structure. These findings suggest that blood-free reperfusion with oxygenated perfluorochemical reduces the area of no reflow through better endothelial cell preservation.

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Reestablishing blood flow to jeopardized myocardium after acute coronary occlusion remains the most effective way to limit myocardial necrosis and improve ventricular function (1–4). However, the beneficial effects of reperfusion after prolonged coronary occlusion may be limited by an incomplete return of blood flow to some areas of the ischemic myocardium, which may result in the conversion of reversibly injured myocytes to irreversible injury (5). This observation has been termed the "no reflow phenomenon." The endothelium of the microvasculature and neutrophilic infiltrates are thought to play a fundamental role in the pathogenesis of the no reflow phenomenon (5,6).

Perfluorochemical (Fluosol-DA 20%, Alpha Therapeutic Corp.), an acellular perfusate, has been shown to reduce infarct size and improve myocardial blood flow (7–11). However, these studies did not measure the area of no reflow and were limited to observational analysis of endothelial injury and suppression of neutrophil infiltration. Furthermore, these studies, utilizing either intracoronary infusion of Fluosol-DA with a patent left main coronary artery or intravenous administration of perfluorochemical, allowed cellular elements, primarily neutrophils, to enter the ischemic bed on reperfusion (9,10).

The aim of this study was to assess the efficacy of blood-free reperfusion with oxygenated Fluosol-DA in limiting no reflow injury in the canine model. In addition, quantitative morphologic techniques were used to determine the degree and extent of endothelial injury and neutrophil plugging of capillaries in the ischemic and nonischemic myocardium.

Methods

This study conformed to the guidelines specified in the "Guide for Care and Use of Laboratory Animals" (DHHS publication no. [NIH] 85-23, revised 1985) and was approved

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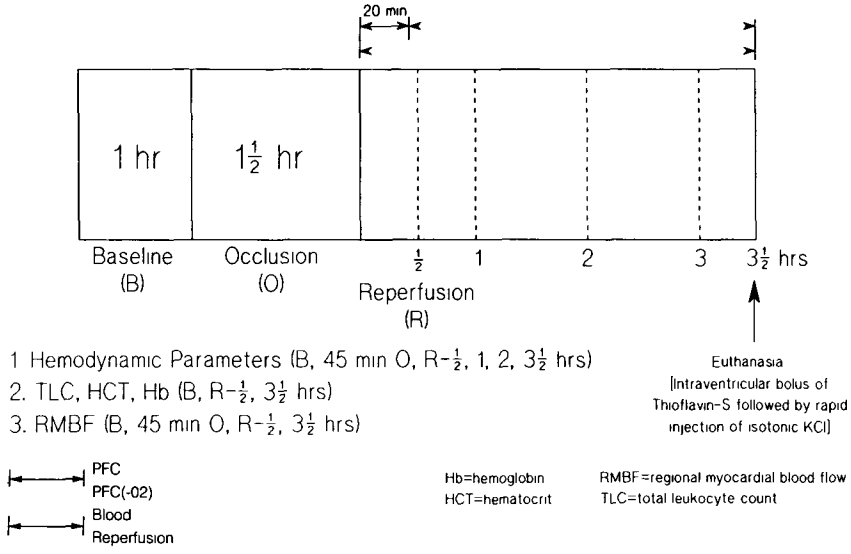

Figure 1. Summary of experimental protocol. PFC and PFC(−O2) = oxygenated and unoxygenated perfluorochemical group, respectively.

by the Armed Forces Institute of Pathology Animal Care and Use Committee.

Experimental protocol (Fig. 1). Male mongrel dogs weighing 15 to 23 kg were used for the study. Dogs were anesthetized with intravenous sodium pentobarbital (25 to 30 mg/kg body weight), intubated with a cuffed endotracheal tube and ventilated with a Harvard positive-pressure respirator. The arterial pH was maintained at 7.40 ± 0.05. An electrocardiogram was continuously recorded on a Holter monitor with a modified Frank lead system. Intravenous morphine sulfate and diazepam were used for supplemental anesthesia during the procedure. Dogs also received intravenous heparin (300 IU/kg). An 8F USCI sheath was inserted into the right carotid artery and 7F USCI sheaths were introduced into the right and left femoral arteries for measurement of arterial blood pressure and withdrawal of blood during blood flow measurements. A baseline blood sample was obtained for total leukocyte count, hemoglobin and hematocrit. All dogs received a 5 ml intravenous bolus injection of reconstituted Fluosol-DA before coronary occlusion, which produced a transient hypotensive episode (50 to 60 mm Hg) in 70% of the dogs. Blood pressure returned to pretest dose levels within 20 min. This finding is predominantly observed in the canine model and has been previously reported (11).

Regional myocardial blood flow was assessed at baseline study before occlusion and serially at 45 min of occlusion and 30 and 210 min of reperfusion. The left coronary ostium was engaged with an 8F USCI guiding catheter, and a 0.014 in. (0.36 cm) flexible steerable guide wire was positioned in the proximal left circumflex coronary artery followed by a 3 mm × 2 cm balloon angioplasty catheter (Advanced Cardiovascular Systems). The angioplasty catheter was used for coronary occlusion, the monitoring of phasic and mean coronary artery pressure (pressure tracings were displayed on a model ES-1000 recorder, Gould) and administration of perfluorochemical. After 45 min of occlusion, hemodynamic measurements were repeated and myocardial blood flow was determined.

Lidocaine (1 mg/kg) was administered as an intravenous bolus injection just before reperfusion. Balloon inflation was maintained for an additional 20 min at the time of reperfusion in dogs treated with perfluorochemical to facilitate blood-free reperfusion. In the control group, the angioplasty balloon was deflated at the time of reperfusion, allowing immediate return of blood flow to the previously ischemic bed. After 20 min of reperfusion, patency of the previously occluded artery was confirmed by restoration of mean coronary blood pressure in the occluded bed.

Intracoronary perfluorochemical infusion. Dogs were randomized before the start of the experiment to one of the following groups: 1) blood-free reperfusion with intracoronary oxygenated perfluorochemical (20 ml/kg per min) for 20 min followed by blood reperfusion (n = 8); 2) intracoronary unoxygenated perfluorochemical administered as in those treated with oxygenated perfluorochemical (n = 8); and 3) blood reperfusion alone (control; n = 8). Fluosol-DA was bubbled with 100% oxygen at a rate of 2 liters/min for 30 min just before infusion. Perfluorochemical was administered into the left circumflex artery through the central lumen of the angioplasty catheter using a Medrad IV power injector. The volume of Fluosol-DA given over the 20 min period was 380 ml for an average weight 19 kg dog. Dogs in the perfluorochemical groups were ventilated with 100% oxygen during the reperfusion period. Regional myocardial blood flow measurements were repeated at 30 and 210 min after reperfusion; at these times, blood samples were obtained for total leukocyte count and hemoglobin and hematocrit determinations. Hemodynamic measurements were repeated at 30, 120 and 210 min of reperfusion.

After the last microsphere injection, the area of impaired perfusion was delineated by an intravenous injection of the fluorescent dye thioflavin-S (1 ml/kg, Sigma). The dye was prepared fresh as a 4% solution in warm saline solution, centrifuged for 10 min at 1,600 rpm to remove particulate matter and injected by hand over 15 to 20 s. Immediately after injection of thioflavin-S, each dog was rapidly eutha-

nized by intravenous administration of isotonic potassium chloride and the heart was excised.

Myocardial blood flow determinations. Transmural myocardial blood flow was assessed with use of colored polystyrene microspheres. Hale et al. (12) demonstrated that myocardial blood flow measured by colored microspheres is comparable with that measured by radioactive tracers. Approximately 5 to 8 × 10^6 colored labeled polystyrene microspheres (yellow, red, green and black), 11.9 ± 1.9 μm (E-Z Trac, SRP), were mixed by vortex agitation for 5 min and hand-injected as a rapid bolus injection through the central lumen of the pigtail catheter placed in the apex of the left ventricular cavity. Left ventricular injections of microspheres are comparable with left arterial injections (13). Reference arterial samples were withdrawn from the femoral artery at a constant rate (13.4 ml/min) with a withdrawal pump (model 901, Harvard Instruments), beginning 5 s before microsphere injection and continuing for 90 s after injection.

At the conclusion of each experiment, the heart was cut in 1 cm slices parallel to the atrioventricular groove, and multiple transmural samples of myocardium (2 to 4 g) were taken from the posterior wall supplied by the left circumflex coronary artery (ischemic region) and the anterior wall (nonischemic region). Samples were further subdivided into epicardial and endocardial halves. Blood and tissue samples were processed with digestion reagents obtained from E-Z Trac, by a modification of a method previously described (12). Ethanol was added to each aliquot of blood and tissue digest and subsequently evaporated for removal of perfluorochemical particles. Final aliquots from the blood and tissue were counted with a hemocytometer, and four to eight chambers were counted for each sample.

Myocardial blood flow (Q_m; ml/min per g) was then calculated as follows: $Q_m = (C_m \times Q_r)/(C_r \times Wt)$, where C_m is the total number of microspheres in the tissue sample, Q_r is the rate of the reference blood withdrawal (ml/min), C_r is the total number of microspheres in the reference blood sample and Wt is the weight of the tissue sample (g).

Measurement of area of impaired perfusion. Thioflavin-S is a fluorescent vital dye (bright yellow-green under ultraviolet light) that stains the endothelium of blood vessels (5). Areas not perfused by thioflavin-S were determined by photographing the myocardial slides under ultraviolet light using high speed Ektachrome film (Kodak). An enlarged tracing (magnification ×5) was made from each slide, and thioflavin-negative and -positive areas were then determined by computer planimetry of tracings by an observer who had no knowledge of the treatment groups.

Histologic analysis. Multiple myocardial biopsy samples were obtained before photographing the heart. Thioflavin-negative and -positive areas from the posterior wall (ischemic region) and anterior wall (nonischemic region) were sampled. Tissue was cut into 1 mm^3 pieces, fixed in McDowell-Trump solution and stored at 4°C for transmission electron microscopy. Specimens were transferred to 1% osmium tetroxide in 0.1 *M* cacodylate buffer, dehydrated and embedded in effapoxy (Fullam Co.). Semi-thin sections (0.5 to 2 μm) were cut, stained with 1% toluidine blue and examined by light microscopy. An average of four biopsy specimens each from the thioflavin-negative and -positive areas of the ischemic myocardium and two specimens each from the nonischemic endocardium and epicardium were obtained. Areas with the most capillaries were selected for ultrathin section cutting, stained with uranyl acetate and lead citrate and examined with a Zeiss 109 IGF electron microscope.

Table 1. Scoring Method of Myocardial and Microvascular Injury

		Myocyte Injury
0	=	Normal myocardium
1	=	Reversible injury
		a) Mild nuclear clumping, mild mitochondrial swelling, prominent I bands
		b) Intracellular edema, moderate nuclear changes, mild to moderate mitochondrial swelling with some separation of cristae
		c) Marked intracellular edema, vacuoles, moderate mitochondrial swelling with occasional mitochondria demonstrating severe swelling, clearing of mitochondrial matrices, occasional focal clumping of cristae
2	=	Irreversible injury
		a) Presence of flocculent densities within mitochondria, including the above changes
		Endothelial Injury
0	=	Normal endothelium
0.5	=	Mild endothelial swelling with decreased pinocytotic vesicles
1.0	=	Moderate endothelial swelling with decreased pinocytotic vesicles
1.5	=	Severe endothelial cell swelling and/or membrane-bound vesicles, myelin figures
2.0	=	Endothelial cell protrusions, membrane-bound vesicles, myelin figures
3.0	=	Red blood stacking with and without endothelial cell swelling
4.0	=	Platelets and fibrin deposition with and without endothelial cell swelling
5.0	=	Leukocyte adhesion with and without endothelial cell swelling
6.0	=	Loss or disintegration of endothelium with and without endothelial cell swelling

Light microscopy. Myocardial sections obtained from the ischemic areas (thioflavin-negative and thioflavin-positive) and the nonischemic endocardial and epicardial (thioflavin-positive) regions were examined under light microscopy by an observer who had no knowledge of other data using an oil immersion objective (100×) and a 10× eyepiece. A total of 200 capillaries were randomly selected from each section and the total number of leukocytes within these capillaries were quantitated. Approximately 90% of the capillaries counted per section were cut in cross section. The remaining were longitudinally cut, and these did not exceed 50 μm in length.

Transmission electron microscopy (Table 1). Twenty consecutive capillaries were photographed from thioflavin-

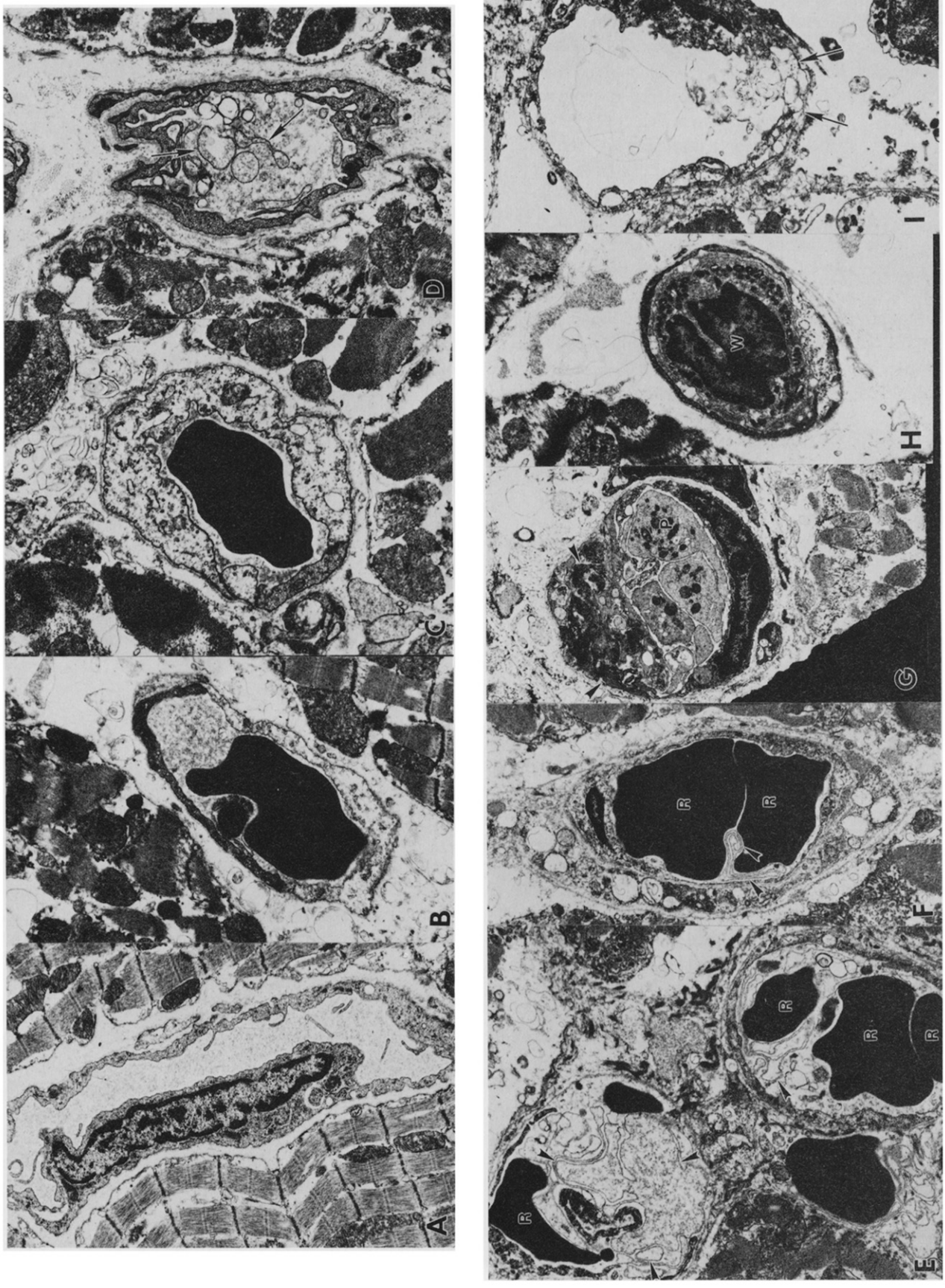
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Figure 2 (opposite page). **A**, Representative electron micrograph showing normal endothelium. **B**, Endothelium showing mild swelling with decreased pinocytotic vesicles. **C**, Endothelium showing moderate cell swelling with a further decrease in pinocytotic vesicles. **D**, Capillary with endothelial projections and membrane-bound vesicles (**arrow**). **E**, Representative capillaries demonstrating endothelial cell protrusions (**arrowheads**) with red cell (R) plugging. **F**, Red cell (R) stacking with focal endothelial protrusions (**arrowheads**) and swelling. **G**, Capillary showing total obstruction by platelets (P) and fibrin deposition (**arrowheads**). **H**, Capillary showing total obstruction by white cells (W). **I**, Capillary with focal endothelial cell disruption (**arrows**).

negative and thioflavin-positive areas along with representative capillaries from nonischemic zones at 3,000× magnification. Capillary and myocyte injury were quantitated (with the observer unaware of treatment) from electron micrographs with use of criteria identified in Table 1 and Figure 2. Each electron micrograph from the appropriate region was assigned a myocyte and endothelial cell score. These values were summed from each region (thioflavin-negative and thioflavin-positive) and divided by the total number of capillaries examined. The percent of capillaries showing endothelial cell injury from the thioflavin-negative and thioflavin-positive regions was also determined.

Statistical analysis. Data are presented as the mean values ± SEM. Serial results among groups were analyzed by repeated measures two-way analysis of variance. If differences were statistically significant, further pairwise analysis was performed by a two-tailed *t* test. Comparisons between groups were analyzed by a nonpaired Student's *t* test with a two-tailed discriminant score. Probability values ≤0.05 were required for an assumption of statistical significance.

Results

Forty dogs underwent coronary occlusion and 16 were excluded from the study. Of these 16 dogs, 7 developed ventricular fibrillation during coronary occlusion (either oxygenated [n = 2] or unoxygenated [n = 1]), perfluorochemical and control (n = 4) dogs and during reperfusion (1 in each treatment group). Six dogs did not develop ST

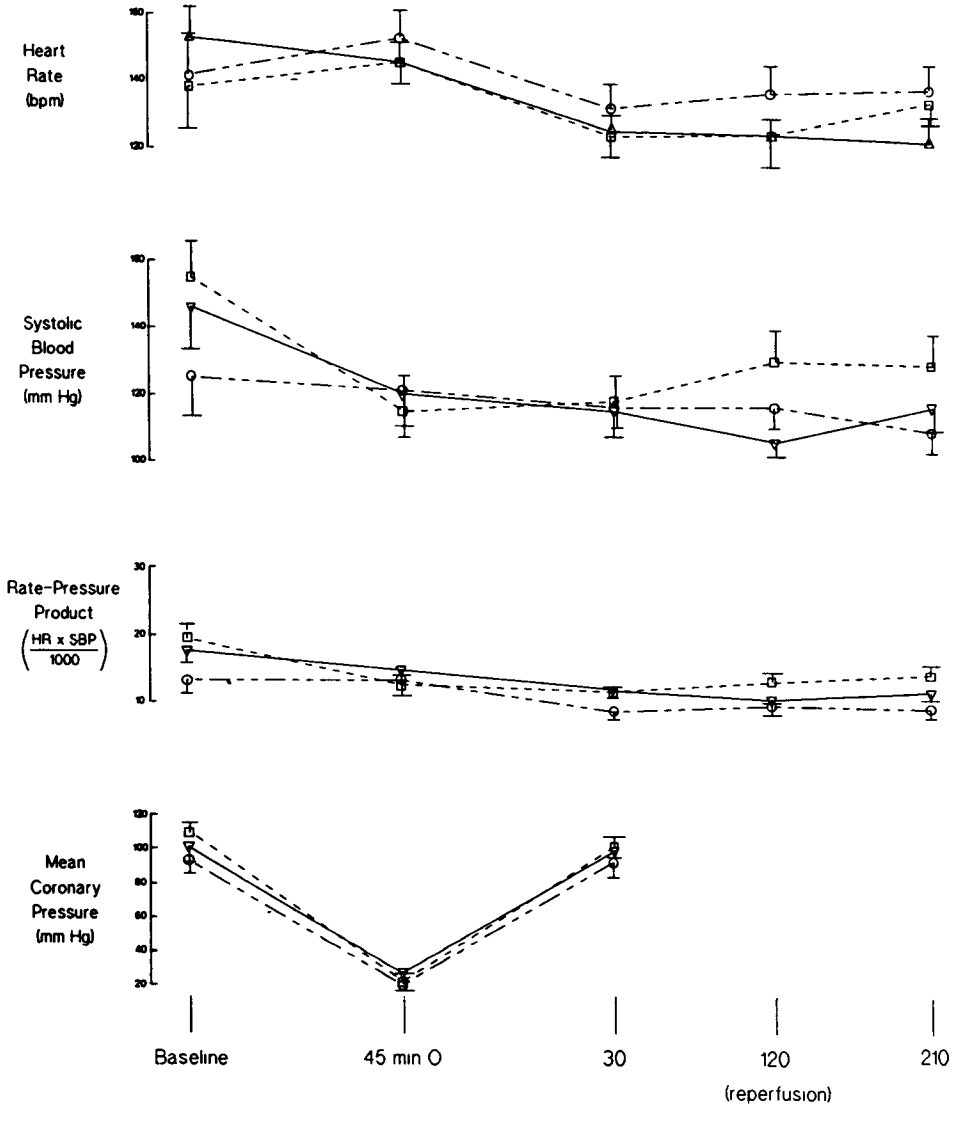

Figure 3. Hemodynamic changes in oxygenated (**triangles**) and unoxygenated (**squares**) perfluorochemical-treated and control (**circles**) dogs during the experimental protocol. No significant differences between groups were noted in any of these variables. HR = heart rate; O = occlusion; SBP = systolic blood pressure.

Table 2. Hematologic Variables in Perfluorochemical-Treated and Control Dogs

	Baseline (ml/min per g)	Reperfusion (ml/min per g)	
		30 min	210 min
TLC ($\times 10^3$/mm^3)			
PFC	5.6 ± 1.6	4.9 ± 0.9	14.1 ± 2.8
PFC($-O_2$)	5.5 ± 0.1	5.4 ± 0.9	10.2 ± 2.4
Cont	5.9 ± 1.0	10.6 ± 2.8	19.8 ± 4.8
HCT (%)			
PFC	41.7 ± 2.6	36.0 ± 1.9	41.8 ± 2.1
PFC($-O_2$)	41.2 ± 1.3	40.4 ± 2.2	43.3 ± 1.7
Cont	36.5 ± 1.5	38.5 ± 1.6	38.7 ± 1.6
Hb (g/dl)			
PFC	14.3 ± 0.5	11.9 ± 0.6	13.7 ± 0.7
PFC($-O_2$)	15.1 ± 0.5	14.3 ± 0.8	15.2 ± 0.5
Cont	12.6 ± 0.4	13.6 ± 0.5	13.6 ± 0.5

Values are expressed as mean values ± SEM. Cont = control blood reperfusion group; Hb = hemoglobin; HCT = hematocrit; PFC = oxygenated perfluorochemical group; PFC($-O_2$) = unoxygenated perfluorochemical group; TLC = total leukocyte count.

segment elevation (≥2 mV) or a significant decrease in coronary blood pressure (≥70% of baseline value). Among the remaining 24 dogs included in the data analysis, 16 underwent blood-free reperfusion with either oxygenated (n = 8) or unoxygenated (n = 8) intracoronary perfluorochemical and 8 control dogs were reperfused with blood.

Hemodynamic variables. Heart rate, systolic pressure, rate-pressure product (an indirect evaluation of myocardial oxygen consumption) and mean coronary pressure are shown in Figure 3. Hemodynamic variables were not significantly changed by intracoronary infusion of perfluorochemical and were similar in all three groups throughout the duration of the study. Mean coronary blood pressure decreased dramatically in all groups at occlusion, whereas at reperfusion, coronary pressure was restored to its baseline value, indicating adequate reperfusion.

Hematologic variables (Table 2). Baseline total leukocyte counts were similar in all groups. An increase in blood leukocytes was noted at 30 min of reperfusion only in control dogs (control group 10.6 ± 2.8 versus 4.9 ± 0.9 min in oxygenated and 5.4 ± 0.9 vs. unoxygenated perfluorochemical groups). By 210 min of reperfusion, total leukocyte count increased in all groups and was maximal in control dogs. Hematocrit and hemoglobin values were similar at all time points except at 30 min of reperfusion in the oxygenated perfluorochemical group, where they were mildly depressed with respect to baseline values. The mean fluorocrit (similar to hematocrit, but applied to the percent of perfluorochemical in the circulating blood) at 30 min of reperfusion was similar in both perfluorochemical groups and varied from 3% to 5%.

Regional myocardial blood flow (Table 3). Baseline blood flow in endocardial and epicardial ischemic areas was similar in all treatment groups. All dogs developed a marked reduction in blood flow in the ischemic endocardial region at 45 min of occlusion, consistent with relatively poor collateral circulation (0.19 ± 0.07, 0.20 ± 0.09 and 0.10 ± 0.03 ml/min per g in the control and oxygenated and unoxygenated perfluorochemical groups, respectively; p = NS). In the epicardial region, flow also decreased during occlusion, but the reduction was less severe than in the endocardium. In the endocardium, blood flow was similar at 30 min of reperfusion in dogs treated with oxygenated perfluorochemical or blood reperfusion alone (1.02 ± 0.05 and 1.00 ± 0.24 ml/min per g, respectively). However, in dogs treated with unoxygenated perfluorochemical, blood flow improved only marginally at 30 min of reperfusion (0.46 ± 0.20 ml/min per g). A significant decrease in blood flow ($p < 0.04$) occurred at 210 min of reperfusion in dogs treated with unoxygenated perfluorochemical or blood reperfusion alone, whereas blood flow in dogs treated with oxygenated perfluorochemical was remarkably preserved.

Effects of perfluorochemical treatment on myocardial perfusion assessed by thioflavin staining. No significant differences were noted in left ventricular weight in all three

Table 3. Regional Myocardial Blood Flow From Endocardial and Epicardial Sections Within the Ischemic Region

	Baseline (ml/min per g)	45 Min Occlusion	Reperfusion (ml/min per g)	
			30 min	210 min
Endocardium				
PFC*	1.12 ± 0.15	0.20 ± 0.09	1.02 ± 0.05	0.97 ± 0.22†
PFC($-O_2$)	0.77 ± 0.01	0.10 ± 0.03	0.46 ± 0.20	0.29 ± 0.06
Cont	1.47 ± 0.15	0.19 ± 0.07	1.00 ± 0.24	0.39 ± 0.08
Epicardium				
PFC*	1.09 ± 0.11	0.25 ± 0.08	1.35 ± 0.05	1.16 ± 0.19
PFC($-O_2$)	0.83 ± 0.20	0.14 ± 0.03	0.65 ± 0.25	0.36 ± 0.04
Cont	1.21 ± 0.24	0.37 ± 0.17	1.22 ± 0.55	0.62 ± 0.26

*Endocardial and epicardial regions contained both thioflavin-negative and -positive areas. †$p < 0.04$ versus unoxygenated perfluorochemical (PFC$-O_2$) and control (Cont) groups. Values are expressed as mean values ± SEM. Myocardial blood flow in the nonischemic region (endocardial and epicardial sections) was similar at all time points (data not shown). PFC = oxygenated perfluorochemical group.

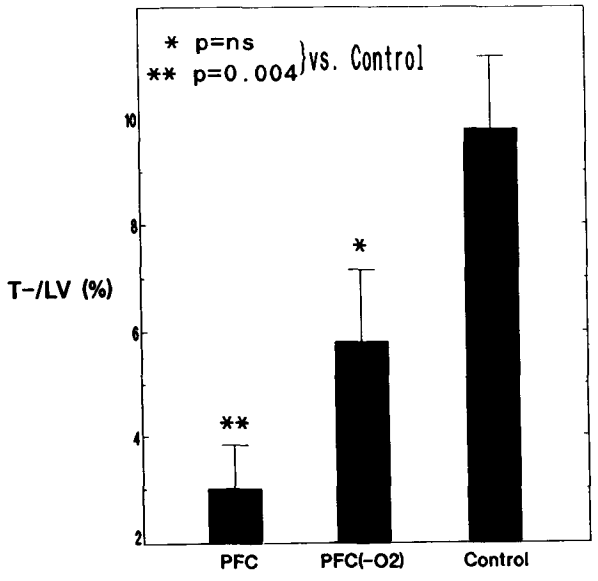

Figure 4. Dogs treated with oxygenated perfluorochemical (PFC) showed a statistically significant decrease in thioflavin-negative areas (T−), when expressed as a percent of the total left ventricle (LV), in comparison with values in control animals. PFC(−O2) = unoxygenated perfluorochemical.

treatment groups (64.5 ± 0.04, 62.1 ± 0.06 and 61.3 ± 0.05 g in control and oxygenated and unoxygenated perfluorochemical groups, respectively). A significant decrease in thioflavin-negative area (expressed as a percent of the left ventricle) was noted in dogs treated with oxygenated perfluorochemical (3.1 ± 1.0%) compared with values in control dogs (9.8 ± 1.6%; $p < 0.04$) (Fig. 4). The size of thioflavin-negative areas was reduced in dogs treated with unoxygenated perfluorochemical (5.7 ± 1.5%) compared with the size in control dogs, but the difference did not reach statistical significance.

Light microscopy (Table 4). A total of 800 capillaries/heart were counted (200/histologic region). The mean number of neutrophils observed in capillaries was significantly greater in thioflavin-negative areas in control dogs (18.9 ± 3.2) than in these areas in dogs receiving oxygenated or unoxygenated perfluorochemical treatment (5.9 ± 1.4 and 5.4 ± 0.8; $p = 0.003$ and 0.002, respectively). No significant differences were noted in neutrophil plugging among the three treatment groups in thioflavin-positive and nonischemic regions (endocardium and epicardium).

Table 4. Quantitation by Light Microscopy of Polymorphonuclear Leukocyte Infiltration per 200 Capillaries Within (Ischemic) Thioflavin-Negative (T−) and Thioflavin-Positive (T+) and (Nonischemic) Endocardial and Epicardial Regions

	Ischemic		Nonischemic	
	T−	T+	Endocardium	Epicardium
PFC	5.9 ± 1.4	4.7 ± 1.8	3.0 ± 0.8	1.4 ± 0.6
PFC($-O_2$)	5.4 ± 0.8	4.0 ± 0.5	1.3 ± 0.6	2.8 ± 0.8
Cont	18.9 ± 3.2*	4.9 ± 1.1	2.7 ± 0.4	2.2 ± 1.0

*$p < 0.003$ versus oxygenated perfluorochemical (PFC) group and $p < 0.002$ versus unoxygenated perfluorochemical (PFC−O_2) group. Values are expressed as mean values ± SEM. Cont = control group.

Table 5. Quantitation of Capillary and Myocyte Injury by Electron Microscopy From Thioflavin-Negative (T−) and Thioflavin-Positive (T+) Areas Within the Ischemic Bed

	Endothelial Cell Injury			
	T−	T+	T+/Myocytes	% Capillary Injury
PFC	1.6 ± 0.2*	1.4 ± 0.2	1.0 ± 0.2	56.1 ± 6.1
PFC($-O_2$)	2.3 ± 0.2	1.4 ± 0.2	1.0 ± 0.2	79.8 ± 5.5
Cont	2.5 ± 0.2	1.3 ± 0.2	1.1 ± 0.4	85 ± 3.8

*$p < 0.05$ versus unoxygenated perfluorochemical (PFC−O_2) and control (Cont) groups. Values are expressed as mean values ± SEM. Endothelial cell injury was quantitated using the morphologic criteria outlined in Table 1. T+/Myocytes refers to a quantitative score of myocyte injury in thioflavin-positive (T+) regions (no injury, score = 0; reversible injury, score = 1; irreversible injury, score = 2). % Capillary Injury refers to the percent of endothelial cells showing injury in thioflavin-negative (T−) regions (score ≥ 0.05). PFC = oxygenated perfluorochemical group.

Electron microscopy (Table 5). Twenty consecutive capillaries from thioflavin-negative and thioflavin-positive areas were photographed and scored by the criteria outlined in Table 1. Thioflavin-negative areas uniformly showed irreversible myocyte injury in all treatment groups. Endothelial cell injury in these areas was maximal in dogs with blood reperfusion alone and was significantly less in dogs treated with oxygenated perfluorochemical than in dogs in either the unoxygenated perfluorochemical or control group (1.6 ± 0.2 vs. 2.3 ± 0.2 and 2.5 ± 0.2, respectively; $p < 0.05$). No significant difference in capillary or myocyte injury was noted in thioflavin-positive areas among all three treatment groups. The percent of capillaries showing endothelial cell injury (mild to severe) was significantly decreased in the group treated with oxygenated perfluorochemical (56.1 ± 6.1%) compared with values in both the unoxygenated perfluorochemical (79.8 ± 5.5%) and control (85.0 ± 3.8%) groups ($p < 0.05$).

Discussion

Reduced no reflow injury after reperfusion with intracoronary oxygenated perfluorochemical. This study demonstrates that blood-free reperfusion with intracoronary oxygenated perfluorochemical administered over 20 min significantly reduces no reflow injury after 90 min of coronary occlusion followed by 210 min of reperfusion. This was evident as a significant reduction in the area of no reflow in dogs treated with oxygenated perfluorochemical compared with values in dogs receiving either unoxygenated perfluorochemical or blood reperfusion alone. Endocardial blood flow in the ischemic zone decreased progressively with reperfusion in control (blood-reperfused) and unoxygenated perfluorochemical groups, but was maintained with oxygenated perfluorochemical. These findings were associated with relative preservation of endothelial cell structure in the

oxygenated perfluorochemical group compared with the unoxygenated perfluorochemical and control groups. Recovery of blood flow in endocardial and epicardial regions in dogs treated with unoxygenated perfluorochemical was less than in control dogs at both 30 and 210 min of reperfusion, possibly a result of an additional 20 min of ischemia.

The number of capillaries containing leukocytes was significantly fewer in dogs treated with perfluorochemical compared with control dogs. These findings support the hypothesis that blood-free reperfusion with oxygenated perfluorochemical improves oxygen delivery to the microvasculature and protects the endothelium from further injury. Fewer leukocytes within capillaries may also have contributed to a decrease in no reflow in both perfluorochemical groups. However, preservation of endothelium was the predominant mechanism in limiting no reflow injury with oxygenated perfluorochemical. One may speculate that the other potential beneficial effects of blood-free reperfusion with perfluorochemical—washout of ischemic metabolites and depressed neutrophil function—are of secondary importance because dogs receiving unoxygenated perfluorochemical did not show a statistically significant reduction in no reflow areas compared with control dogs. However, it must be recognized that dogs in the unoxygenated perfluorochemical group were exposed to an additional 20 min of ischemia.

Endothelium and reperfusion injury. The role of endothelium during reperfusion has long been of interest. Although early reperfusion reduces infarct size, improves ventricular function and reduces the incidence of death (14–16), the introduction of oxygen and blood elements (especially neutrophils) into the ischemic myocardium has been reported (17–20) to induce deleterious effects in the microvascular bed. We hypothesize that endothelial injury may be the critical factor in the no reflow phenomenon. Moreover, the degree of endothelial injury appears to be dependent on the duration of coronary occlusion. Kloner et al. (5), using thioflavin-S as a marker, were unable to demonstrate no reflow after 40 min of coronary occlusion, but 90 min resulted in a marked heterogeneity of blood flow to the previously ischemic myocardium. Histologic analysis of no reflow areas revealed numerous structural abnormalities in endothelial cells (5).

The emphasis on endothelial injury as the basis of the transformation of reversibly injured myocytes into irreversibly injured cells during reperfusion is in contrast to earlier studies (21) with permanent coronary occlusion, which showed that myocyte injury precedes microvascular injury. However, not all endothelial cells within the ischemic region are affected at the same time or to a similar extent. Kloner et al. (6) also reported that ultrastructural irreversible myocyte injury becomes evident in the subendocardium by 40 min of ischemia; however, capillary changes were not seen until 60 to 90 min of ischemia. At 60 min of coronary occlusion, endothelial changes in capillaries were present in approximately 20% of vessels, and by 90 to 180 minutes, 40% of vessels showed microvascular injury (6). In the present study, 85% of capillaries showed some degree of endothelial injury after 90 min of coronary occlusion followed by 210 min of blood reperfusion. Therefore, although it appears that capillary injury lags behind myocyte injury, there is considerable overlap.

We postulate that myocardial salvage with reperfusion should occur in areas where myocardial cells are reversibly injured and contain a relatively intact microvasculature. After reperfusion, reversibly injured myocytes may be converted to irreversibly injured cells, and the ischemic microvasculature probably plays a fundamental role through endothelial cell swelling, vascular plugging by cellular blood elements or explosive myocyte swelling that is capable of compressing adjacent capillaries (5,19).

Ambrosio et al. (22) observed a progressive decrease in postischemic blood flow after 90 min of coronary occlusion and 210 min of reperfusion. This correlated with large perfusion defects identified by lack of thioflavin-S staining. Our observations were similar with 210 min of reperfusion in the control dogs (with blood reperfusion). Because myocardial blood flow was comparable during coronary occlusion in all three treatment groups in our study, myocyte ischemic injury must also have been similar before reperfusion. Earlier studies with perfluorochemicals in our laboratory (9,10) measured serial changes in myocardial blood flow in the ischemic myocardium with use of both xenon-133 and radioactive microspheres. Myocardial blood flow at reperfusion was greater in dogs treated with perfluorochemical than in those treated with crystalloid therapy; however, these data were limited to measurement of flow only up to 60 min of reperfusion (9,10). The present study extends these observations to 210 min of reperfusion and demonstrates a significant decrease in the no reflow areas with oxygenated perfluorochemical.

Perfluorochemicals (mechanism of action). Perfluorochemicals have been shown to reduce infarct size and improve contractile function in various models of occlusion and reperfusion (7–10,23). However, the precise mechanism involved in limiting reperfusion injury is still not known. Because the perfluorochemical Fluosol-DA has a small particle size (1 to 2 μm) and low viscosity, it would be expected to increase oxygen delivery to ischemic tissue (24). In reperfusion, perfluorochemical may provide oxygen to areas of the microcirculation inaccessible to red blood cells as a result of anoxia and red and white cell sludging. Indeed, enhancement of myocardial oxygen delivery by perfluorochemical has been shown in the central ischemic zone in a model of permanent occlusion (24).

We previously demonstrated (7–10) that large and intermediate doses of perfluorochemical administered intravenously or by the intracoronary route significantly reduced neutrophil infiltration in models of permanent and temporary occlusion. In addition, neutrophil chemotaxis and lysosomal enzyme release were also markedly suppressed with perfluorochemical ex vivo (10). In the present study, leukocytosis was delayed in addition to a decrease in capillary plugging by

neutrophils with perfluorochemical treatment compared with blood reperfusion (control) treatment. Although the number of leukocytes in capillaries was decreased similarly in both oxygenated and unoxygenated perfluorochemical-treated groups with respect to values in the control group, no significant differences were noted in thioflavin-negative areas between unoxygenated perfluorochemical-treated and control groups. We hypothesize that leukocyte infiltration and capillary plugging in the reperfused myocardium are inhibited by perfluorochemical; oxygenation of perfluorochemical offers the additional advantage of endothelial preservation. Together, these effects on leukocytes and endothelial cells may be crucial factors in the limitation of reperfusion injury by perfluorochemical.

Limitations of the study. Several considerations need to be discussed before acceptance of the methods in the present study. The canine infarct model is predisposed to a high degree of collateral circulation, with marked variation among dogs. In the present study, myocardial blood flow during coronary occlusion was similar among all three treatment groups. Therefore, we assumed that all dogs were subjected to similar degrees of ischemia. We did not normalize thioflavin-negative areas as a percent of the area at risk. Previous canine studies (8,10,11) have shown that infarcted myocardium, when expressed as a percent of the area at risk, correlates well with myocardial blood flow. These studies (8,10,11) also demonstrated significant infarct size reduction when the infarcted area was expressed as a percent of the left ventricle. In addition, colored dyes normally used to measure risk regions may interfere with the fluorescence staining imparted by thioflavin-S. Finally, although lidocaine has been reported (25) to reduce infarct size, in the present study, lidocaine was administered in equivalent concentrations in all three treatment groups and therefore its effects, if any, should have been equally distributed.

Conclusions. Preservation of the ischemic myocardium with oxygenated perfluorochemical may limit myocardial necrosis by decreasing infarct extension during reperfusion. Our results show that the oxygenated perfluorochemical Fluosol-DA 20% may hold considerable promise in reducing reperfusion injury and may be a useful adjunct therapy to thrombolytic agents in the treatment of myocardial infarction in patients. Improvement of myocardial perfusion through capillary preservation in previously ischemic tissue may also facilitate the delivery of other cardioprotective drugs.

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